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Decreased Cortisol Awakening Response after Early Loss Experience

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Summary

Early loss experience (ELE) due to death or separation is a major risk factor for the development of several psychiatric and physical disorders in adulthood. Few studies have focused on the effects of ELE on neuroendocrine systems, which might mediate this risk in part. The goal of this study was to evaluate salivary cortisol responses to awakening in individuals with and without ELE. A total of 95 healthy college students (29 men, 66 women) completed a questionnaire on ELE and were instructed to collect saliva immediately after awakening and 30 minutes later. Fifty-five of the 95 subjects reported having experienced the separation or

divorce of their parents and/or the death of a close relative before the age of 14 years. Subjects with such ELE exhibited decreased salivary cortisol responses to awakening compared to subjects without ELE (net increase: 4.78 nmol/l versus 9.83 nmol/l; t_{93}=2.88, p=.005). The effect was most pronounced in individuals who experienced multiple types of ELE, while there were no sex differences. In conclusion, ELE appears to be associated with decreased salivary cortisol responses to awakening. Low cortisol awakening responses are believed to reflect altered dynamics of the hypothalamic-pituitary-adrenal (HPA) axis, possibly conferring risk for certain stress-related disorders.

[200 words]

Key words: Childhood loss, parental separation, salivary cortisol

Introduction

At the beginning of the last century, Sigmund Freud had recognized that experiences of loss early in life are linked to the later manifestation of melancholia (Freud, 1957). This seminal observation was subsequently confirmed in a large number of epidemiological, twin and case-control studies demonstrating that loss of a parent due to death or separation increases the risk for major depression in adulthood (Agid et al., 2000). Early loss experience (ELE) was also found to predispose for a variety of other psychiatric disorders, including bipolar disorder, anxiety disorders, schizophrenia, and alcohol abuse (Agid et al., 1999; Kendler et al., 1992, 2002a,b). There is also evidence for a relationship between ELE and physical illnesses, functional somatic syndromes and several health risk behaviors (Lowman et al., 1986; Felitti et al., 1998; Agid et al., 1999).

There is abundant evidence from research in rodents and non-human primates that maternal separation early in development induces persistent alterations of brain circuits involved in the mediation of stress and emotion, leading to altered neuroendocrine responsiveness and behavioral changes (Ladd et al., 2000; Sánchez et al., 2001). While maternal separation in rodents has been associated with increased stress responsiveness, findings in non-human primates exposed to maternal or social deprivation are less consistent with some studies reporting increased (Higley et al., 1991, 1992; Fahlke et al., 2000) and others reporting decreased (Clarke, 1993; Lyons et al., 1999) basal or stress-induced cortisol output. Of note, decreased cortisol excretion particularly in the early morning hours has been reported for marmoset monkeys exposed to repeated maternal separation (Dettling et al., 2002). Given the important physiological and behavioral effects of cortisol in the organism's adaptation to the environment, such changes might plausibly confer vulnerability to various pathologies, likely in the context of genetic risk.

Although there is a multitude of clinical studies documenting changes of the hypothalamic-pituitary-adrenal (HPA) axis in maltreated children and adults with childhood abuse histories (DeBellis et al., 1999; Teicher et al., 2002; Heim et al., 2004), surprisingly few studies have focused on the neuroendocrine effects of ELE. In one study, children adopted from Romania who spent more than eight months in their first years of life in an orphanage demonstrated increased salivary cortisol concentrations 15 to 30 minutes after awakening when compared to control children (Gunnar et al., 2001). In another study in children, the diurnal decline in salivary

cortisol concentrations was found to be negatively correlated with out-of-home placements and the extent of emotional deprivation, suggesting elevated diurnal cortisol secretion (Kaufman, 1991). In contrast, Carlson and Earls (1997) report that severely socially deprived children show significantly lower salivary cortisol concentrations at 8 a.m. in the morning compared to home-reared children. Studies in adults have provided some evidence for HPA axis hyperactivity after ELE. Breier et al. (1988) measured increased basal cortisol levels in a single afternoon blood sample obtained from adults with early parental loss who had a history of psychopathology. Both psychopathology and cortisol concentrations were positively associated with the quality of life and social support after the loss. Another recent study reported that adult men with ELE due to parental death exhibit increased salivary cortisol concentrations at various time points throughout the day compared to controls, when aggregating measures over 5 consecutive days. The most robust difference was detected at 8 a.m. in the morning and the effect was not due to increased depression or anxiety (Nicolson, 2004). Finally, in a study by Luecken (2000), adults with early parental loss experiences who had a poor relationship with the surviving parent, showed increased cortisol responses during a speech stressor. Interestingly, adults with other types of adversity, i.e. child abuse, also exhibit increased stress responses (Heim et al., 2000b), but cortisol levels in the morning are low (Heim et al., 2001), similar to some findings in deprived non-human primates or severely deprived children (Dettling et al., 2002; Carlson and Earls, 1997).

Importantly, with the exception of the study by Gunnar et al. (2001), the above studies reporting either increased or decreased early morning cortisol levels in ELE collected samples at fixed time points, e.g. at 8 a.m., and did not consider potential effects of different times of awakening on the results. It is well known that there is a rapid rise of cortisol in response to awakening that peaks after approximately 30 minutes. The cortisol awakening response is considered a reliable marker of the dynamics of the HPA axis and demonstrates moderate to high within-subject stability (Pruessner et al., 1997; Wuest et al., 2000; Edwards et al., 2001). The awakening response appears to be independent from diurnal cortisol secretion and cortisol responses to stress, though it is correlated with the response to standard ACTH adrenocorticotropin stimulation, suggesting that the cortisol awakening response is a marker for adrenocortical reactivity (Schmidt-Reinwald et al., 1999; Edwards et al., 2001). In clinical studies, the cortisol awakening response has been found to be increased in euthymic patients with a history of major depression (Bhagwagar et al., 2003) and persons with high

neuroticism (Portella et al., 2004), possibly reflecting risk for depression. Decreased cortisol awakening responses have been reported for subjects with chronic stress (Schulz et al., 1998), burnout (Pruessner et al., 1999), chronic pain (Geiss et al., 1997) or PTSD posttraumatic stress disorder (Rohleder et al., 2004), and other health problems (Kudielka and Kirschbaum, 2003). The morning cortisol response has never been studied in adults with early adverse experience. Altered cortisol awakening responses as a function of early adversity, such as ELE, might convey risk for one or another of the above disorders and might be a useful marker to detect risk for these disorders after ELE.

In addition to the above considerations, it remains largely unknown to what extent dispositional factors, such as gender, interact with early adversity, such as ELE, in influencing adult HPA axis function, thereby potentially moderating disease vulnerability. It has been reported that girls are more vulnerable to develop depression after parental loss than boys (Kunugi et al., 1995). Because the neuroendocrine stress response is subject to sex differences (Rhodes and Rubin, 1999), one might speculate that differential disease risk after ELE might be associated with different neuroendocrine consequences of ELE in boys and girls.

The goals of the present study were 1) to assess salivary cortisol responses to awakening in a sample of healthy college students stratified based on ELE and 2) to assess potential sex differences in the neuroendocrine correlates of ELE.

Method

Subjects

A total of 95 healthy subjects, including 29 men and 66 women aged 19 to 36 years (mean age=21.7, SD=2.87), participated in the study. All subjects were undergraduate students in Psychology at the University of Trier, Germany, and were recruited during classes. The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from subjects prior to the study.

Questionnaires

Experiences of loss and other types of early adversity were assessed using a translated version of the Childhood Traumatic Events Survey (Pennebaker and Susman, 1988). The instrument screens for childhood adverse experience in six questions. The age limit of the questionnaiore is 17; however we modified the age limit to consider only events before the age of 14 years. We chose the age of 14 years as a cut-off to approximate the onset of puberty. Subjects indicate whether or not a particular event occurred and at what age it occurred. Subjects also rate the subjective burden of the event. The questionnaire includes two questions on loss, i.e. "Before the age of 14, did you experience the death of a very close friend or family member?" and "Before the age of 14, was there a major upheaval between your parents, such as divorce or separation?". Subjects who responded "yes" to any of these 2 questions were considered to have experienced ELE. It should be noted that ELE included the death of persons other than a parent, i.e. relatives or friends, if the person was considered "very close" by the subject. We collected additional information on the relationship to the deceased person and found that loss due to death was mostly limited to relatives in the present sample. Only one person had experienced parental loss.

To control for the impact of depression and trait anxiety on cortisol awakening responses, we administered the German versions of the Beck Depression Inventory (BDI; Hautzinger et al., 1994) and the trait anxiety scale of the Spielberger State-Trait Anxiety Inventory (STAI; Laux et al., 1981). We also collected information on smoking habits, use of oral contraceptives, and quality and duration of sleep in the night before the sampling to control for potential confounds.

Saliva Sampling

Saliva samples were collected at home using a commercially available sampling device (Sarstedt, Rommelsdorf, Germany). Subjects were instructed to collect saliva samples immediately (0 minutes) after awakening and 30 minutes later on a week day. We chose to collect at 2 time points only to diminish subject burden. Previous studies have shown that the net cortisol increase between 0 and 30 minutes after awakening is highly sensitive to group differences (e.g., Pruessner et al., 1999). Subjects were instructed to not brush their teeth and to abstain from breakfast and smoking until the end of the sampling period. Subjects returned the samples to the laboratory where they were stored at -20ºC until assayed

Cortisol Assay

Saliva samples were thawed and spun at 3000 rpm for 5 minutes to obtain 0.5-1.0 ml clear saliva with low viscosity. The free cortisol concentration in saliva was measured using a time-resolved immunoassay with fluorescence detection, as described previously (Dressendoerfer et al., 1992). Intra and inter assay variability was less than 10 and 12 percent, respectively.

Statistical Analyses

Groups were stratified according to the presence (ELE) or absence of ELE (non-ELE). Demographic and psychological variables were compared using two-tailed t-tests. The net increase of cortisol secretion after awakening was calculated by subtracting the concentration measured directly after awakening from the concentration measured after 30 minutes. Group differences were analyzed using two-tailed t-tests. To identify potential sex-differences, two-way analysis of variance (ANOVA) was performed with Sex and ELE as factors. Cortisol awakening responses between subgroups of ELE and controls were compared using ANOVA and Least Square Difference (LSD) post-hoc testing. Correlations between the cortisol awakening response and demographic or psychological variables were calculated using the Spearman rank correlation. For all analyses, the level of significance was set at 0.05.

Results

Early Loss Experience and Psychological Variables

Of the 95 the participants, 11 subjects reported having experienced both, the death of a close relative and the separation or divorce of their parents before the age of 14 years. Thirty-one subjects had experienced the death of a close relative but no divorce or separation of their parents before the age of 14 years. Thirteen had experienced the separation or divorce of their parents. These three groups were collapsed into the ELE group (N=55). The remaining subjects reported no significant ELE (non-ELE, N=40). There were no significant differences between the ELE and non-ELE groups respect to mean age, sex distribution, BDI scores and STAI trait scores. The overall mean BDI score was 6.68 (SEM=0.66) and the overall mean STAI trait score was 51.63 (SEM=0.98). Both mean scores are in the normal range. The mean age at first ELE was slightly lower in subjects with ELE in form of death of a relative as well as parental divorce or separation compared to subjects with only one type of ELE, but this effect did not reach statistical significance. The demographics and psychological characteristics in the sample, stratified by type of loss experience, are presented in Table 1.

Cortisol Awakening Response

Subjects with ELE demonstrated a significantly reduced net cortisol awakening response when compared non-ELE subjects [4.78 (SEM=1.04) nmol/l versus 9.83 (SEM=1.47) nmol/l; t_{93}=2.878, p=.005]. There were no group differences in cortisol concentrations at 0 minutes (see Figure 1). We next subdivided the ELE group according to the type of loss into three groups (separation/divorce, death of relative, or both) and compared these groups to the non-ELE group (see Figure 2). There was a significant difference between the four groups in terms of net cortisol increases after awakening ($F_{3,91}$=2.928, p=.038). Subjects with both types of loss experiences (separation/divorce and death of relative) showed the lowest cortisol awakening response. Post-hoc analysis confirmed significantly different cortisol awakening responses between subjects with multiple ELE and non-ELE subjects, as well as between subjects with ELE in the form of the death of a relative and the non-ELE group. There was no statistical difference in cortisol awakening responses between subjects with ELE due to parental separation/divorce and the non-ELE group, possibly due to lack of power.

Sex Differences

The decrease in cortisol awakening responses in ELE subjects compared to non-ELE subjects was more pronounced in women than in men (Women: ELE: 3.94 [SEM=1.17] nmol/l versus non-ELE: 10.54

[SEM=1.93] nmol/l ; Men: ELE: 6.85 [SEM=2.14] nmol/l versus non-ELE: 8.35 [SEM=2.19] nmol/l). However, ANOVA did not reveal a statistically significant interaction effect of sex and ELE.

Confounding Variables

There were no significant influences of age, smoking habits, use of oral contraceptives, and quality and duration of sleep in the night before the sampling on cortisol awakening responses. It should be noted that four subjects reported having experienced sexual abuse and one subject reported having experienced severe physical abuse before the age of 14 years. Three of these five subjects also reported ELE. The two subjects with early abuse experience but no ELE had markedly lower cortisol awakening responses than subjects without early abuse experiences, which were comparable to the mean of the ELE group. Because of the low sample size, no statistical comparisons were performed.

Comment

We here report the finding that experiences of loss early in life are associated with decreased cortisol responses to awakening in healthy college students. The effect was most pronounced in subjects who experienced both the death of a close relative and parental separation or divorce before the age of 14 years, likely reflecting more profound or severe disruption of the family environment early in life. It should be emphasized that only one of the subjects included in this study had lost a parent due to death. However, even the comparatively moderate experience of loss of a relative due to death by itself was associated with decreased cortisol awakening responses. The effect of decreased cortisol awakening responses did not attain statistical significance for subjects who exclusively experienced the separation or divorce of their parents before 14 years of age, probably due to lack of power, as the mean net awakening cortisol was about the same as the response of subjects who experienced the death of a relative alone. Although it appeared that the decrease in the cortisol awakening response in the ELE group was more pronounced in women than in men, the interaction did not attain significance as well. However, it might be worthwhile to test the hypothesis of gender differences in the neuroendocrine effects of ELE, potentially leading to differential risk for pathology, in larger samples in the future. Such studies should employ more extensive assessments of HPA axis function using dynamic challenge tests after early adversity, given the known gender differences in HPA regulation and

the stress responses (Rhodes and Rubin, 1999).

Our findings are generally comparable to some findings in non-human primates exposed to repeated maternal separation (Dettling et al., 2002) as well as findings in severely socially deprived Romanian orphans (Carlson and Earls, 1997), both demonstrating decreased morning cortisol levels. The findings are also generally similar to reports of decreased morning cortisol levels in women with child abuse experience, suggesting that different types of early adversity might be associated with similar HPA axis alterations (Stein et al., 1997; Heim et al., 2001). Our findings are not in accord with findings by Nicolson (2004) who reported increased salivary cortisol concentrations at 8 a.m. in adult men with parental loss. However, none of the above studies assessed cortisol concentrations relative to awakening, and it is therefore difficult to compare and interpret similarities and differences. The one study that assessed cortisol levels relative to awakening after ELE was performed in children and revealed increased cortisol levels at 15 to 30 minutes after awakening. It is unknown what factors might contribute to these differences. Potential factors include differences in neuroendocrine regulation after ELE as a function of developmental stage, differential timing of the ELE, different severity of the ELE, and different concomitant and subsequent psychosocial conditions to name a few. Future neuroendocrine studies will have to address the multiple facets of early loss, or early adversity in general, in relation to other risk factors and developmental time courses as well as moderator and mediator variables.

Although there is a known link between early loss and depression, awakening cortisol responses in euthymic patients with lifetime depression as well as in highly neurotic subjects, who are at risk for depression, are increased (Bhagwagar et al., 2003; Portella et al., 2004). HoweverIn contrast, we found decreased awakening cortisol responses in subjects with ELE,. This appears to be in contrast with even though it has well-established findings that severe early loss been shown that severe loss experiences increases the risk for major depression (Agid et al., 2000). It might be speculated that the rather mild to moderate type of ELE in our sample does not increase risk for depression and, accordingly, our subjects were not depressed or highly anxious. In fact, our finding that moderate ELE is related to decreased cortisol awakening responses, bears similarity with findings in a number of pre-clinical and clinical conditions, including states of chronic stress and burnout (Pruessner et al., 1999), chronic pain (Geiss et al., 1997), but also some clinical conditions such as

posttraumatic stress disorder (Rohleder et al., 2004). Early Early life adversity is an established risk factor for all of these conditions. Given that we observed a similar pattern in a healthy group of students with moderate ELE, one might hypothesize that low cortisol awakening responses reflect risk to develop such disorders rather than representing correlates of the disordered state. Future studies should evaluate whether different type, timing and severity of early adversity leads to differential neurobiological patterns, thus explaining variability in pathological outcomes.

There is some evidence that the cortisol awakening response might reflect adrenocortical capacity and is not related to the neuroendocrine cortisol responses to acute psychosocial laboratory stress response (Schmidt-Reinwald et al., 1999). We therefore suggest that decreased cortisol awakening responses might be a marker of adrenaocortical dysfunction, rather than changes at higher levels of HPA regulation. A relative decreased adrenal capacity might indeed be a vulnerability factor for certain somatic and mental syndromes, due to a potential lack of regulating effects of cortisol on metabolic, immune and central nervous systems (Heim et al., 2000a). For example, a decreased adrenal capacity and therefore reduced cortisol secretion availability of to cortisol adrenocorticotropin may might lead to lack of regulatory control a disinhibition of the immune system, thus increasing risk for disorders characterized by fatigue and pain. At the central nervous system level, reduced availability of cortisol responses and hence to deleterious effects of the immune system. However, it should be noted that a reduced cortisol secretion to adrenocorticotropin might also have adaptive consequences, for example by preventing memory impairment after long-term exposure to high endogenous levels of glucocorticoids (Lupien et al., 2005). might promote activation of neural circuits implicated in stress and PTSD (Heim et al., 2000a; Yehuda, 2002). In addition, there is evidence that the awakening response is also influenced by the suprachiasmatic nucleus in the hypothalamus (Thorn et al., 2004), and dysregulations in this nucleus might be of importance to certain disorders, such as chronic fatigue syndrome or depression.

A number of limitations of this study should be noted. First, we recruited a convenience sample of healthy college students. This sample might not be representative of the general population. Also, results might have been different in a clinical sample with potentially more severe early losses and psychopathology. It should be noted that we chose a very broad definition of ELE that included rather mild to moderate loss experiences that are likely not 'traumatic'. None Only one of the subjects had lost a parent due to death. One might question

whether the types of loss studied in this sample, such as loss of a relative, reflect normalcy of human childhood rather than exceptional loss events. However, the inclusion of mild loss experiences would lead to underestimation rather than overestimation of the neuroendocrine correlates of loss. Furthermore, as mentioned before, the observed effect was largely carried by subjects who had experienced multiple types of losses, probably reflecting more severe family disruption, which might be outside of the normal range. It is limitation of the study that we did not include additional measures to address general family environment and parental or other social relationships in that might have mediated or moderated the observed effects. Moreover, unfortunately we did not measure the time of awakening of the subjects. Recently, it has been found that substantial differences in wake-up time can have an impact on the cortisol awakening response (see Clow et al., 2004). However, reported differences in cortisol awakening responses due as a function of early versus late awakening (3 hours difference in awakening time; to the time of awakening Kudielka & Kirschbaum, 2003) that are reported in the literature, are considerably much smaller than differences in cortisol responses that we our observed differences due to as a function of presence or absence of ELE. Together with the fact that subjects in the present study did not report sleep difficulties, we suggest that (Kudielka & Kirschbaum, 2003), indicating that our findings could cannot be alternatively explained by potential discrepancies in awakening -up time, even if ample differences in time of awakening would be present between subjects with and without ELE. . A final limitation of the study is the fact that we could not control for the subjects' compliance with the saliva sampling protocol. Studies using collection devices equipped with electronic sensors that monitor collection times revealed that the compliance with instructions is generally low (Kudielka et al., 2003). To alleviate this limitation, college students were questioned regarding their compliance when they delivered the samples and samples of subjects who reported non-compliance were not included in this report.

Despite of these limitations, the current data provide evidence that ELE is associated with persistent alterations of cortisol responses to awakening, which is a stable marker of adrenal function that has been associated with a variety of clinical disorders related to stress. Many of these disorders are more prevalent in women than in men. Further studies should evaluate the neuroendocrine consequences of ELE and other adversities across the lifespan, as well as their interaction with dispositional factors. Such studies might shed light on pre-disease

pathways that convey vulnerability to a variety of disorders and might lead towards the identification of targets for prevention of such pathology in children who suffered loss and other adversities early in their lives.

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Table 1: Description of demographics and psychological characteristics of all subjects and subgroups stratified by type of loss experience

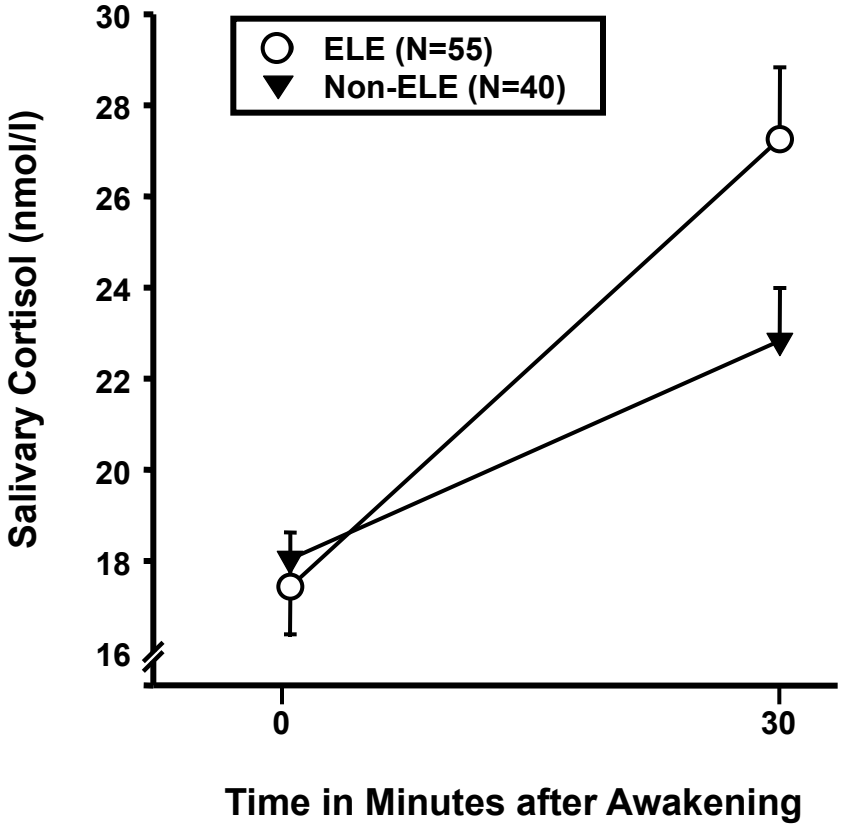
Subject characteristic	All subjects (N=95)	Divorce or separation (N=13)	Death of relative (N=31)	Both types of ELE (N=11)	No ELE (N=40)	Statistics
Mean age in years (SEM)	21.7 (0.29)	22.1 (1.03)	22.1 (0.65)	22.8 (0.69)	21.0 (0.28)	F=1.78, df=3,94, NS
Female sex in %	69.5	92.3	58.1	81.8	67.5	χ^2=5.96, df=3, NS
BDI score (SEM)	6.68 (0.66)	8.54 (2.09)	4.71 (0.72)	5.91 (1.64)	7.83 (1.20)	F=1.84, df=3,94, NS
STAI trait T score (SEM)	51.63 (0.98)	51.54 (2.25)	50.81 (1.69)	50.09 (2.77)	52.73 (1.62)	F=0.34, df=3,94, NS
Mean age at first ELE (SEM)	---	8.23 (0.82)	7.48 (0.60)	5.09 (0.97)	---	F=3.15, df=2,54, p=0.51

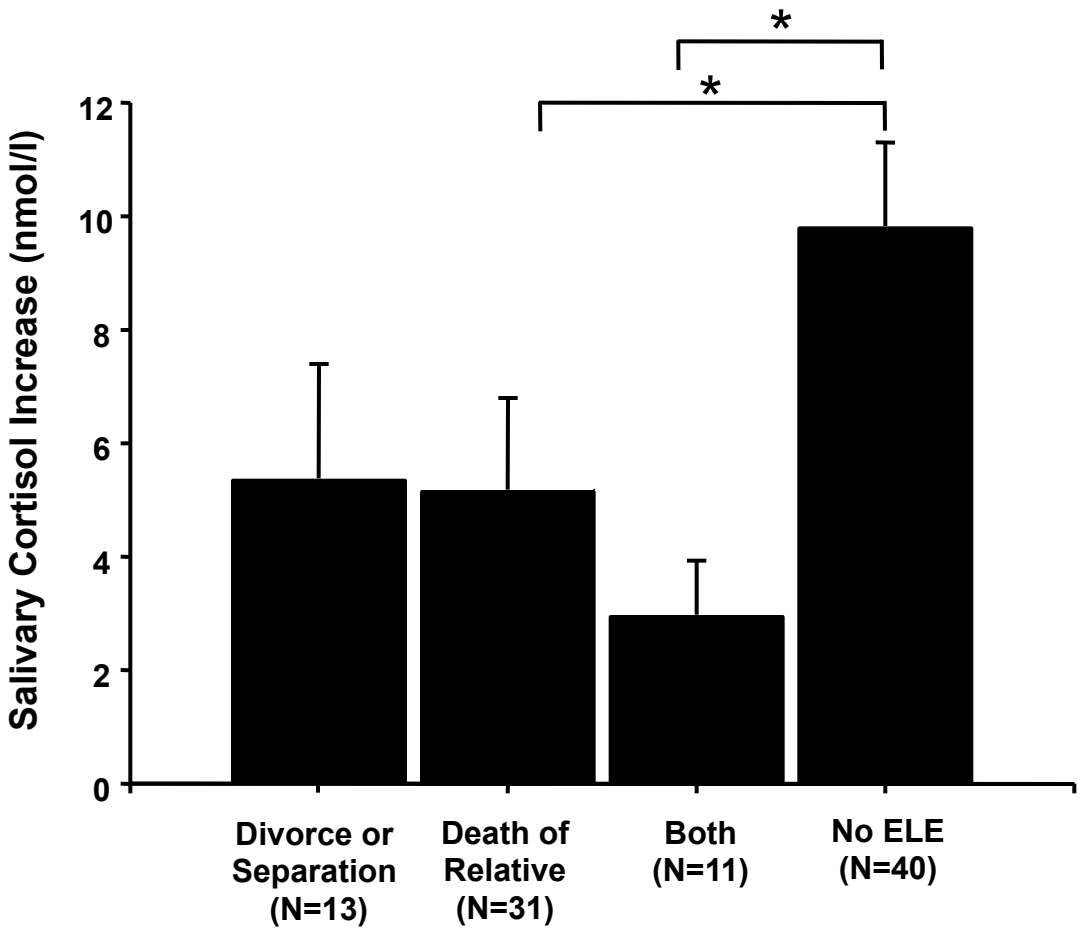
Abbreviations: BDI, Beck Depression Inventory; STAI, State-Trait Anxiety Inventory; ELE, early loss experience; NS, not significant

Figure Legends

Figure 1: Salivary cortisol concentrations (means ± SEM) at 0 minutes and 30 minutes after awakening in male and female subjects with early loss experience (ELE; N=55) versus male and female subjects without early loss experience (non-ELS; N=40). The mean net increase of cortisol concentrations (30 minutes concentration minus 0 minutes concentration) was significantly lower in the ELE group versus controls (t_{93}=2.878, p=.005).

Figure 2: Salivary cortisol awakening responses (means ± SEM) in four groups: 1) early loss experience (ELE) due to separation or divorce of parents, 2) ELE due to death of a relative, 3) both types of ELE, and 4) no ELE. Analysis of variance revealed significant differences between groups effect ($F_{3,91}$=2.928, p=.038). ∗ p<0.05 versus no-ELE


ELE (N=55)
Non-ELE (N=40)
Salivary Cortisol (nmol/l)
30
28
26
24
22
20
18
16
0
30
Time in Minutes after Awakening


*
*
12
10
8
6
4
2
0
Salivary Cortisol Increase (nmol/l)
Divorce or Separation (N=13)
Death of Relative (N=31)
Both (N=11)
No ELE (N=40)